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A Phase I Trial of Autologous Cytokine-Induced Killer Cells for the Treatment of Relapsed Hodgkin Disease and Non-Hodgkin Lymphoma

Thomas Leemhuis, Sandra Wells, Christian Scheffold, Matthias Edinger, Robert S. Negrin

Division of Bone Marrow Transplantation, Stanford University Medical Center, Stanford, California

Correspondence and reprint requests: Robert S. Negrin, MD, CCSR 2205, 269 West Campus Dr., Stanford University, Stanford, CA 94305 (e-mail: negrs@stanford.edu).

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ABSTRACT

We have previously reported on the ex vivo generation of cytotoxic effector cells, termed cytokine-induced killer (CIK) cells, that have both in vitro and in vivo antitumor activity in murine models. We now report on our efforts for the large-scale expansion of CIK cells and also present preliminary results from a phase I clinical trial. Nine patients with advanced Hodgkin disease (n = 7) and non-Hodgkin lymphoma (n = 2), all of whom had relapsed after an autologous transplantation, were treated with escalating doses of CIK cells (3 patients at each dose level of 1×10^9, 5×10^9, or 1×10^{10} cells). The CIK cells were produced by culturing unselected cells from steady-state apheresis products with interferon γ, OKT3, and interleukin 2. After 21 days in culture, with the addition of fresh media and interleukin 2 every 3 to 4 days, the median culture was 97% viable (range, 61%-100%), 98% $CD3^+$ (range, 66%-99%), 76% $CD8^+$ (range, 27%-96%), 23% $CD4^+$ (range, 6%-78%), 20% $CD3^+CD56^+$ (range, 8%-58%), and <1% $CD16^+56^+$ (range, 0.2%-7.7%). The $CD3^+CD56^+$ cells have previously been shown to exhibit the most cytotoxic activity. The absolute number of $CD3^+CD56^+$ cells typically expanded 290-fold (range, 3- to 4000-fold) under these culture conditions. In vitro cytotoxic activity was measured against a human B-cell tumor line (OCI-Ly8). At a 40:1 effector-target cell ratio, CIK cells killed 32% (range, 2%-69%) of the target cells. A total of 21 infusions were administered to 9 patients. The number of CIK cells infused ranged from 1.0×10^9 to 1.0×10^{10} per treatment. Toxicity was minimal, and there were no immediate adverse reactions to the infusions. Two patients had partial responses, and 2 patients had stabilization of disease: 1 for more than 18 months. Considering that these were heavily pretreated patients with advanced hematologic malignancies, we believe that CIK cells expanded in this fashion may have utility for the treatment of high-risk patients with evidence of minimal residual disease after autologous transplantation.

KEY WORDS

Cytotoxic cells • Adoptive immunotherapy • Cell expansion

INTRODUCTION

Relapse is a major cause of treatment failure after autologous hematopoietic cell transplantation. After transplantation, most patients achieve a state of minimal residual disease in which alternative treatment strategies such as immunotherapeutic interventions may hold promise. A variety of strategies have been explored in an effort to activate autologous effector cells to recognize and kill tumor targets. Interleukin (IL)–2–activated natural killer (NK) cells or lymphokine-activated killer (LAK) cells [1] are produced by short-term culture of lymphocytes in the presence of high concentrations of IL-2. These cells have the cell-surface phenotype of $CD3^-CD56^+$. The clinical application of these cytotoxic effector cells has been limited by their relatively low antitumor cytotoxic activity and the difficulty encountered in obtaining clinically relevant cell numbers [2,3]. In addition, LAK cells require IL-2 for biological activity; this has been associated with considerable toxicity [4] that complicates interpretation of potential efficacy.

Tumor-infiltrating lymphocytes (TILs), which are cultured and expanded from tumor tissue, are another

strategy. These cells are $CD8^+$ and recognize tumors through T-cell receptor (TCR)–mediated mechanisms. TILs are challenging to expand and may not be recoverable from patients who have minimal tumor bulk. After infusion of TILs, tumor responses have been noted in some patients [5]. An alternative strategy has been to expand peripheral blood mononuclear cells in the presence of interferon (IFN)–γ, IL-2, and a monoclonal antibody against the CD3 surface antigen (OKT3). We have characterized the resulting effector cell populations that share functional and phenotypic properties of both T cells and NK cells. These cells have been termed cytokine-induced killer (CIK) cells. CIK cells exhibit non–major histocompatibility complex–restricted killing of tumor cell targets both in vitro and in vivo [6,7]. The cell population with the greatest cytotoxic activity in these cultures expresses both the CD3 marker characteristic of T cells and the CD56 marker characteristic of NK cells. CIK cells are predominantly $CD8^+$ and TCR-α/β. $CD3^+CD56^+$ T cells circulate in the peripheral blood and have cytotoxic activity [8]. CIK cells are unique in that they have phenotypic and functional attributes of both T cells and NK cells. They are functionally like NK cells in that they do not require priming, yet they are also like T cells in that they express CD3 and are rapidly expandable in culture.

CIK cells are capable of lysing a variety of tumor cell lines and fresh tumor isolates in vitro and have greater activity in vivo than LAK cells. CIK cells can be expanded from patients with a variety of hematologic malignancies and are capable of lysing fresh autologous myeloid leukemias [9,10]. CIK cells do not require exogenous administration of IL-2 for in vivo activity. In one study, approximately 40% to 50% of severely compromised immunodeficient mice injected with CIK cells after they were challenged with a B-lymphoma cell line (SU-DHL4) became long-term survivors, compared with none of the control animals and none of the animals treated with LAK cells [7]. In another study, expanded CIK cells from patients with chronic myeloid leukemia (CML) suppressed autologous CML colony growth in vitro but did not inhibit the growth of normal hematopoietic colonies. CIK cultures were completely Philadelphia negative after 4 weeks in 12 of 13 cultures. [11]. Autologous CIK cells were capable of inhibiting CML growth in severely compromised immunodeficient mice engrafted with CML. [11] The in vivo activity of CIK cells against HeLa cells injected into severely compromised immunodeficient mice was measured by using a novel bioluminescence assay to visualize tumor growth [12] Mice receiving 1 or 2 treatments of 1×10^7 CIK cells showed statistically significant reductions in tumor signal compared with control animals.

A similar population of murine cells is readily expandable from bone marrow and spleen. Murine cells similarly express CD3, αβTCR, and the NK cell markers NK1.1 and DX5 [13]. Murine cells were capable of controlling the syngeneic tumor growth of Bcl_1 lymphoma cells. It is interesting to note that the expanded murine cells had a markedly attenuated capacity for graft-versus-host disease (GVHD) induction related to the production of IFN-δ [13].

CIK cells function through primarily perforin-mediated mechanisms [14]. CIK cells do not express CD16 (Fc receptor) and therefore do not kill targets via antibody-dependent cellular cytotoxicity. A cytoplasmic granule release mechanism is involved, and there are at least 2 distinct activation pathways that can cause degranulation, because anti-CD3–mediated degranulation is inhibited by immunosuppressive agents (cyclosporin A and FK506), whereas target cell–stimulated degranulation is not [15]. Both forms of activation are known to cause the release of perforin, a pore-forming serine esterase enzyme. The cytotoxic effects of CIK cells can be blocked by antibodies against the intercellular adhesion molecules LFA-1 and intercellular adhesion molecule 1 [6]. More recently, a major role of NKG2D engagement has been demonstrated in which blocking monoclonal antibodies and small interfering RNA studies inhibiting NKG2D expression abrogated cytotoxicity [16].

On the basis of these in vitro and in vivo antitumor biological activities, a phase I clinical trial was performed to test the safety and feasibility of infusing autologous CIK cells into patients with relapsed Hodgkin disease (HD) and non-Hodgkin lymphoma (NHL). The purpose of this trial was 2-fold: (1) to test the feasibility of culturing clinically relevant numbers of CIK cells in a clinical laboratory setting and (2) to determine the toxicity associated with infusing CIK cells into cancer patients.

MATERIALS AND METHODS

Clinical Trial Design

This trial was designed as a dose-escalation study. The study followed a dose-escalation format of 3 CIK cell dose levels with 3 patients at each dose level. Dose escalation was allowed if there were no grade III or IV toxicities at a given dose. The study was initiated at 1×10^9 cells per infusion, and the dose was escalated to 5×10^9 and finally to 1×10^{10} cells per infusion. Up to 3 infusions were allowed, assuming that there was no toxicity and no evidence of disease progression. The dose infused in subsequent infusions was at the dose level open at that particular time. Eligible patients had either B-cell NHL or HD and had relapsed after conventional therapy. All patients had undergone autologous hematopoietic cell transplantation and had relapsed. A total of 9 patients were treated, the clinical characteristics of which are shown in Table 1.

Table 1. *Clinical Responses*

Patient No.*	Age (y)	Sex	Diagnosis	Dose ($\times 10^9$)†	Response
1	28	M	HD	1,1	NR
2	67	M	HD	1,5,5	PR
3	53	M	HD	1,5	PR
4	45	M	NHL	5,5	NR
5	25	M	NHL	5,5,5	SD
6	23	M	HD	5,10,10	NR
7	30	F	HD	10,10	NR
8	32	M	HD	10,10,10	SD
9	30	F	HD	10	NR

HD, indicates Hodgkin disease; NHL, non-Hodgkin lymphoma; NR, no response; PR, partial response; SD, stable disease.
*Nine patients were treated with a total of 21 CIK infusions.
†Patients received 1, 2, or 3 separate infusions approximately 2 months apart at the doses indicated.

CIK cells were generated by culturing a steady-state apheresis product for 21 days, as described below. The cell dose infused was defined by protocol and was based on the total viable cell number. Patients were followed up for signs of toxicity (as defined by World Health Organization toxicity criteria) immediately after and for 2 months after infusion. Although the purpose of this trial was primarily to determine feasibility and safety, the patients were also followed up with regular physical examinations, biopsies, and radiographic studies to document objective tumor responses.

Only patients with pathologically confirmed relapsed HD or NHL of B-cell origin with no alternative curative potential were eligible to be enrolled into this study. The patients were required to be <65 years of age and to have signed an informed consent form according to Stanford University Institutional Review Board guidelines. They were not to have received any chemotherapy for 3 weeks before the start of apheresis. Their leukocyte count had to be at least 3000/μL, and their platelet count had to be at least 100 000/μL before apheresis. Patients required evidence of adequate renal and hepatic function and a Karnofsky performance score of >70 before being admitted into this study.

CIK Cell Expansion

Starting with a steady-state apheresis product (Cobe Spectra; Gambro BCT, Lakewood, CO), unfractionated cells were cultured overnight at 2×10^6 total nucleated cells per milliliter in Aim V (Gibco BRL, Grand Island, NY) with 10% autologous plasma (collected at the time of apheresis) and IFN-γ 1000 U/mL (InterMune Pharmaceuticals, Palo Alto, CA). The Aim V media contained gentamycin 10 μg/mL and streptomycin 50 μg/mL. To maintain a closed system, the cells were incubated in Lifecell tissue culture bags (Baxter Oncology, Deerfield, IL). Most of the cultures were initiated with a total of 2.4×10^9 cells in a total of 1200 mL of media divided equally among 6 culture bags. After 18 to 24 hours in culture at 37°C and 5% CO_2, OKT3 50 ng/mL (Ortho Biotech, Raritan, NJ) and IL-2 300 U/mL (Chiron, Emeryville, CA) were added. IL-2 was added every 3 to 4 days, regardless of cell concentration, and fresh medium was added as needed to maintain a cell concentration of 1 to 4×10^6/mL. A sterile connecting device (Terumo, Somerset, NJ) was used to attach tubing as needed for cytokine and media additions.

Each of the individual culture bags was sampled on day 19 for sterility testing, and then, on day 21, the cells were harvested and washed twice with a COBE 2991 (Gambro BCT) cell washer. The cells were resuspended to approximately 300 mL in Normosol (Hospira, Lake Forest, IL) and 1% human serum albumin. Additional samples were removed for sterility testing, phenotypic analysis, and cytotoxicity assays, and the cells were infused within 60 minutes of harvesting. The cell dose was defined by the protocol and was based on the total viable cell number.

Quality Control Assays

Cell dose criteria were based on the total viable cell number, as determined by manual hemacytometer cell counts and trypan blue dye exclusion. Each culture bag was tested for sterility 2 days before pooling (day +19). Small aliquots (1 mL) were cultured for aerobic bacterial and fungal cultures. Preliminary reports were generated at 24 hours so that only the culture bags that showed no growth were pooled together for harvest. Samples from the pooled product were also submitted for sterility testing (5-day culture), but results were not available before infusion.

Phenotypic analysis of the preculture and postculture cell populations was achieved by using fluorescein-labeled monoclonal antibodies to CD3 and CD16 and phycoerythrin-labeled antibodies to CD56, CD4, and CD8 (Becton Dickinson, San Jose, CA). Cells were incubated with antibodies for 30 minutes at 4°C. Excess antibody was removed, and the stained cells were analyzed with a FACScan instrument (Becton Dickinson).

In vitro cytotoxicity assays were performed on the

day of infusion by using a chromium 51 (^{51}Cr) release assay. Target cells (OCI-Ly8) were labeled with ^{51}Cr (Dupont-NEN, Boston, MA) by incubating 2×10^6 cells per milliliter in RPMI with 300 μCi of ^{51}Cr for 2 hours at 37°C. The labeled cells were washed 3 times with phosphate-buffered saline and then resuspended to 2×10^5 cells per milliliter in RPMI/10% fetal calf serum. A total of 2×10^4 labeled cells (0.1 mL) were placed into the appropriate number of 96-well microtiter plate wells. Effector cells were added at a 40:1 effector-target ratio and incubated at 37°C for 4 hours. At the completion of the assay, an aliquot (75 μL) of the supernatant was counted with a gamma counter. The percentage of specific ^{51}Cr release was calculated according to the following equation:

$$\% \text{ specific release} = \frac{(\text{CPM test}) - (\text{CPM spontaneous release})}{(\text{CPM maximal release}) - (\text{CPM spontaneous release})} \times 100$$

Spontaneous release was obtained by incubating cells in media alone, and maximal release was obtained after treatment with 2% NP-40.

Clinical Response Criteria

For the purposes of this study, response criteria were as follows: complete response indicated complete resolution of all clinical and radiographic signs of disease; partial response indicated measurable radiographic tumor reduction; stable disease indicated neither reduction nor progression of disease; and no response indicated continue progression of disease.

RESULTS

The patients' median age was 30 years (range, 23-67 years). Seven of the 9 patients treated had HD, and 2 patients had NHL. Five of the 7 HD patients were male, as were both of the NHL patients. All patients had experienced treatment failure with prior therapies, including autologous hematopoietic cell transplantation. The target CIK cell dose was achieved in 19 of 21 cultures. Both cultures that fell short of target cell numbers were from the highest-dose cohort. Overall, a median 7-fold total cell expansion was observed during 21 days in culture (range, 1- to 11-fold), meaning that most often excess cells were produced. Typically, the viable cell number would decline slightly during the first few days in culture but would increase rapidly after 5 to 7 days. The culture bags were usually split into 2 bags on day 12 to 14 to maintain the desired cell concentration. The median postculture viability was 97.5%.

A total of 197 culture bags were used (including subcultures) to produce enough CIK cells for the 21 infusions. On 4 occasions (2%), a positive sterility test obtained from the day 19 (or day 20) sample affected the pooling decision. Three of these 4 occurrences could have been false positives, on the basis of the growth pattern observed and of the lack of macroscopic contamination evidence in the culture itself; however, none of the bags was pooled for infusion. Only once did a culture show growth after the 24-hour test period and, therefore, after infusion.

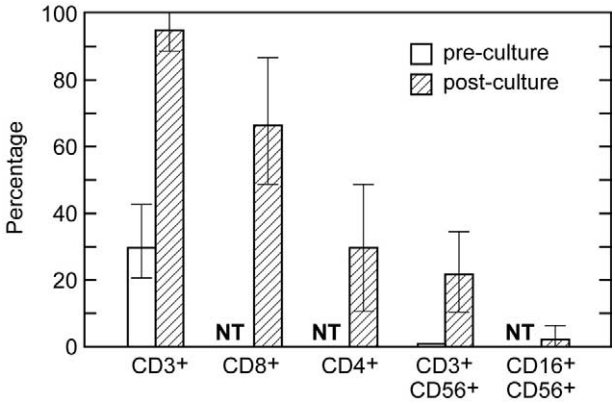

Figure 1. Phenotypic analysis (mean ± SD) of CIK cultures before and after the 21-day culture period. NT indicates not tested.

Results from phenotypic analysis of the CIK cultures are presented in Figure 1. These cultures started with a typical $CD3^+$ T-cell percentage of approximately 30%, whereas the starting $CD3^+CD56^+$ percentage was <1%. After the 21-day culture period, the mean CD3 percentage was 95.3% (range, 66.2%-99.6%), and only 4 of 21 cultures contained <95% $CD3^+$ cells. On average, 67% of the $CD3^+$ cells were $CD8^+$, and 30% were $CD4^+$. The median percentage of $CD3^+CD56^+$ cells was 22% (range, 8%-58%). In contrast, traditional $CD16^+CD56^+$ NK cells comprised only approximately 2% of the postculture cell population.

Expansion of $CD3^+$ T cells was measured by flow cytometry. Figure 2 shows the results for each of the 21 individual cultures, grouped according to patient number. The total CD3 number expanded a median of 24-fold under these culture conditions (range, 2- to 73-fold). Similarly, $CD3^+CD56^+$ cells typically expanded 290-fold (Figure 3), but there was a large variation in the level of expansion observed (range, 3- to 4000-fold). The reason for the variability is unclear, although it is likely that prior patient treatment played a role, as did the difficulty in accurately measuring such low starting percentages. There was less individual patient variability among cultures than among patients (Figures 2 and 3).

The CIK cells were tested in a ^{51}Cr release assay for their in vitro cytotoxicity against a neoplastic B-cell line (OCI-Ly8) immediately after their 21-day

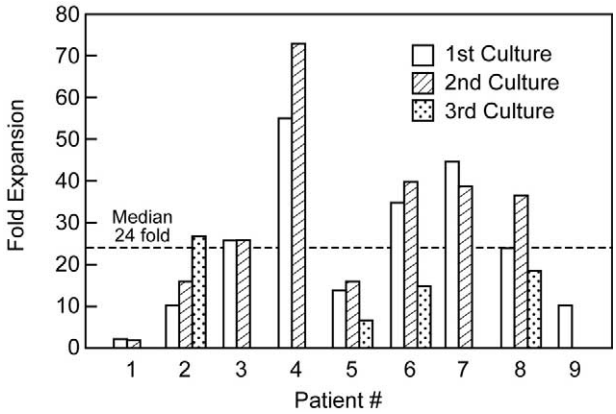

Figure 2. Expansion of $CD3^+$ cells during 21 days in culture, as described in Materials and Methods. A total of 21 cultures were initiated with steady-state apheresis products from 9 different patients. Fold expansion = absolute number of $CD3^+$ cells at the end of 21 days in culture/absolute number of $CD3^+$ cells at the start of culture.

culture period. The results are shown in Figure 4. At a 40:1 effector-target ratio, the CIK cells typically killed 32% of the target cells in 4 hours (range, 2%-69%). Some of the patients' CIK cells lysed these target cells better than other patients' cells; however, multiple cultures from the same patient had similar in vitro cytotoxic activity. Thus, with 1 exception (patient 8), the variability seems more patient related than individual culture related.

Minimal infusion-related toxicity was observed. One case of asymptomatic mild hypotension occurred immediately after infusion, and it was quickly resolved. One patient developed fever (38°C), and 1 patient experienced thrombocytopenia that was thought to be related to disease progression rather than to treatment.

Although clinical response was only a secondary end point for this particular study, those results are shown in Table 1. Of the 9 patients treated, 2 patients had partial responses (measurable radiographically documented tumor reduction), and 2 patients had stabilization of their disease—1 for more than a year. There was no measurable response in the remaining 5 patients. All clinical responses were transient in this heavily pretreated cohort of patients with extensive disease.

DISCUSSION

Autologous hematopoietic cell transplantation has proven to be an effective therapy for patients with a variety of hematologic malignancies. However, a significant percentage of patients ultimately relapse even though most patients enter a state of minimal residual disease. Therefore, strategies effective at treating patients with minimal disease are likely to improve overall survival. A variety of treatment strategies have been explored, including cytokines [17], activated NK cells [18], and monoclonal antibodies [19].

In this study, we used ex vivo–expanded CIK cells to evaluate the feasibility and toxicity of this treatment approach. CIK cells have several advantage that makes this cellular product attractive: they are readily expandable from patients with a variety of malignancies [9] and have in vivo activity superior to that of LAK cells [7], without the need for exogenous administration of IL-2.[7,11] CIK cells have been well tolerated in animal models of both human[11,12,20] and murine[21,22] disease. CIK cells were expanded from patients with either HD or NHL who had experienced treatment failure with prior autologous transplantation. All patients had extensive and measurable disease. Cell expansion was similar to that obtained from buffy coat samples obtained from healthy donors [7]. Total cell, $CD3^+$, and $CD3^+CD56^+$ expansions were variable from patient to patient; however, individual patients had similar growth characteristics from culture to culture. Expansion of $CD3^+CD56^+$ T cells, which have been identified as the cellular population with the greatest in vitro cytotoxicity, was 290-fold after 21 days in culture. Most patients reached their protocol-defined cell dose, which varied from 1×10^9 to 1×10^{10} cells.

Recently we have identified NKG2D as the major cell-surface receptor involved in CIK cell–mediated cytotoxicity because monoclonal antibodies directed against this cell-surface molecule and small interfering

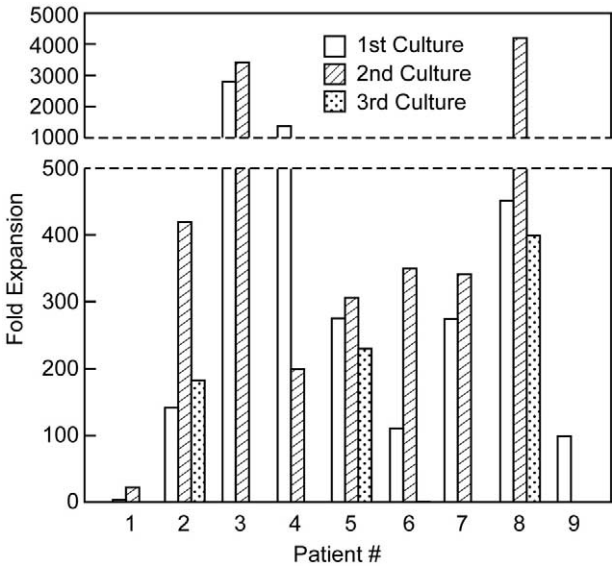

Figure 3. Expansion of $CD3^+CD56^+$ cells during 21 days in culture, as described in Materials and Methods. Twenty of 21 cultures (from 9 different patients) that were initiated with steady-state apheresis products were tested. Fold expansion = absolute number of $CD3^+CD56^+$ cells at the end of 21 days in culture/absolute number of $CD3^+CD56^+$ cells at the start of culture.

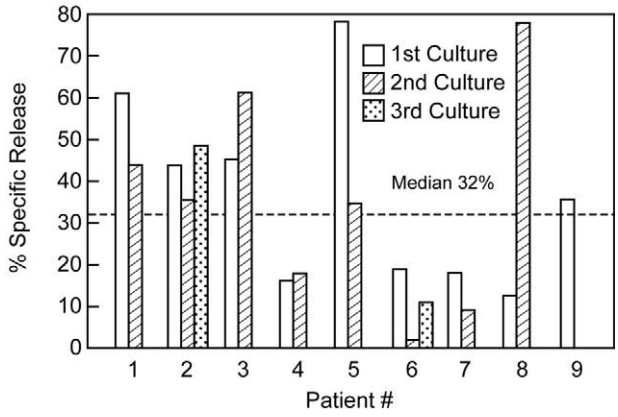

Figure 4. Cytotoxicity of expanded CIK cells. CIK cells from each culture were tested in ^{51}Cr-release cytotoxicity studies against the B-cell non-Hodgkin lymphoma cell line OCI-Ly8, as described in Materials and Methods. Results are shown for an effector-target ratio of 40:1 for each culture.

RNA–containing sequences of the C-terminal portion of the gene decrease NKG2D expression; both result in a significant reduction in cytotoxic function. Although $CD3^+CD56^+$ cells have the highest level of expression of NKG2D, which increases with in vitro culture, $CD8^+$, but not $CD4^+$, T cells also express NKG2D [16]. It is interesting to note that over time, upregulation of the adaptor molecule Dap10 correlates with cytotoxic activity. These studies would predict that tumor cells expressing ligands for NKG2D would be the most likely to respond to CIK-mediated immunotherapy.

Toxicity was mild after the infusion of 21 cellular products to 9 patients. One patient developed mild hypotension, which was asymptomatic and responded to intravenous fluids, and a second patient developed a low-grade fever. One additional patient developed thrombocytopenia several weeks after the first CIK cell infusion; this was thought to be related not to the treatment, but rather to progression of the underlying HD. Therefore, the CIK cell infusions were well tolerated.

Although it was not the intention of the study to evaluate responses, 2 patients had radiographic improvement, with either a reduction or resolution of pulmonary nodules evaluated by serial computed tomographic scanning. An additional 2 patients had stabilization of their disease—in 1 patient for over a year. There was no clear relationship of disease response to cell dose; however, all patients who experienced either a partial response or stabilization of disease (patients 2, 3, 5, and 8) had in vitro cytotoxic activity above the median. The number of patients treated was too small to reach definitive conclusions.

A similar population of cells has been evaluated clinically in another study of patients with solid tumors, in whom minimal toxicity was also observed [23]. This larger randomized study evaluated cells derived from patients with hepatocellular carcinoma after surgical resection expanded in a similar fashion. It is interesting to note that the patients who received the cells had a statistically significant reduction in postsurgical disease recurrence [24].

A similar population of murine CIK cells that are derived by identical culture conditions has also been evaluated in murine models of GVHD and graft versus leukemia. These expanded cells were capable of graft-versus-leukemia effects with attenuated GVHD [13,14].

In summary, CIK cells are readily expandable from patients after transplantation, and cells sufficient for clinical application can be obtained in a routine fashion. After infusion, toxicity was minimal, and preliminary evidence of disease response was observed. The infusion of CIK cells may be a useful treatment strategy to attempt to reduce the risk of relapse in high-risk patients after autologous transplantation.

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REFERENCES

1. Rosenberg SA, Lotze MT, Muul LM, et al. Observations on the systemic administration of autologous lymphokine-activated killer cells and recombinant interleukin-2 to patients with metastatic cancer. *N Engl J Med.* 1985;313:1485-1492.
2. Lafreniere R, Rosenberg SA. Successful immunotherapy of murine experimental hepatic metastases with LAK cells and rIL-2. *Cancer Res.* 1985;45:3735-3741.
3. Takhashi J, Nakada T, Puisieux I. Inhibition of human colon cancer growth by antibody-directed human LAK cells in SCID mice. *Science.* 1993;259:1460-1463.
4. Siegel JP, Puri RK. Interleukin-2 toxicity. *J Clin Oncol.* 1991; 9:694-704.
5. Rosenberg SA, Packard BS, Aebersold PM, et al. Use of tumor-infiltrating lymphocytes and interleukin-2 in the immunotherapy of patients with metastatic melanoma. A preliminary report. *N Engl J Med.* 1988;319:1676-1680.
6. Schmidt-Wolf IG, Lefterova P, Mehta BA, et al. Phenotypic characterization and identification of effector cells involved in tumor cell recognition of cytokine-induced killer cells. *Exp Hematol.* 1993;21:1673-1679.
7. Lu PH, Negrin RS. A novel population of expanded human CD3+CD56+ cells derived from T cells with potent in vivo antitumor activity in mice with severe combined immunodeficiency. *J Immunol.* 1994;153:1687-1696.
8. Ortaldo JR, Winkler-Pickett RT, Yagita H, Young HA. Comparative studies of CD3− and CD3+ CD56+ cells: examination of morphology, functions, T cell receptor rearrangement, and pore-forming protein expression. *Cell Immunol.* 1991;136: 486-495.
9. Alvarnas JC, Linn Y-C, Hope EG, Negrin RS. Expansion of cytotoxic $CD3^+CD56^+$ cells from peripheral blood progenitor

cells of patients undergoing autologous hematopoietic cell transplantation. *Biol Blood Marrow Transplant.* 2001;7:216-222.
10. Linn YC, Lau LC, Hui KM. Generation of cytokine-induced killer cells from leukaemic samples with in vitro cytotoxicity against autologous and allogeneic leukaemic blasts. *Br J Haematol.* 2002;116:78-86.
11. Hoyle C, Bangs CD, Chang P, Kamel O, Mehta B, Negrin RS. Expansion of Philadelphia chromosome-negative CD3(+)CD56(+) cytotoxic cells from chronic myeloid leukemia patients: in vitro and in vivo efficacy in severe combined immunodeficiency disease mice. *Blood.* 1998;92:3318-3327.
12. Sweeney TJ, Mailänder V, Tucker AA, et al. Visualizing the kinetics of tumor-cell clearance in living animals. *Proc Natl Acad Sci U S A.* 1999;96:12044-12049.
13. Baker J, Verneris MR, Ito M, Shizuru JA, Negrin RS. Expansion of cytolytic $CD8^+$ NK-T cells with limited capacity for GVHD induction due to interferon-γ production. *Blood.* 2001; 97:2923-2931.
14. Verneris MR, Ito M, Baker J, Arshi A, Negrin RS, Shizuru JA. Engineering hematopoietic grafts: purified allogeneic hematopoietic stem cells plus expanded CD8+ NK-T cells in the treatment of lymphoma. *Biol Blood Marrow Transplant.* 2001;7: 532-542.
15. Mehta BA, Schmidt-Wolf IG, Weissman IL, Negrin RS. Two pathways of exocytosis of cytoplasmic granule contents and target cell killing by cytokine-induced CD3+ CD56+ killer cells. *Blood.* 1995;86:3493-3499.
16. Verneris MR, Karami M, Baker J, Jayaswal A, Negrin RS. Role of NKG2D signaling in the cytotoxicity of activated and expanded CD8+ T cells. *Blood.* 2004;103:3065-3072.
17. Nagler A, Ackerstein A, Or R, Naparstek E, Slavin S. Immunotherapy with recombinant human interleukin-2 and recombinant interferon-alpha in lymphoma patients postautologous marrow or stem cell transplantation. *Blood.* 1997;89:3951-3959.
18. Benyunes MC, Massumoto C, York A, et al. Interleukin-2 with or without lymphokine-activated killer cells as consolidative immunotherapy after autologous bone marrow transplantation for acute myelogenous leukemia. *Bone Marrow Transplant.* 1993;12:159-163.
19. Horwitz SM, Negrin RS, Blume KG, et al. Rituximab as adjuvant to high-dose therapy and autologous hematopoietic cell transplantation for aggressive non-Hodgkin lymphoma. *Blood.* 2004;103:777-783.
20. Schmidt-Wolf IG, Negrin RS, Kiem HP, Blume KG, Weissman IL. Use of a SCID mouse/human lymphoma model to evaluate cytokine-induced killer cells with potent antitumor cell activity. *J Exp Med.* 1991;174:139-149.
21. Baker J, Verneris MR, Ito M, Shizuru JA, Negrin RS. Expansion of cytolytic CD8(+) natural killer T cells with limited capacity for graft-versus-host disease induction due to interferon gamma production. *Blood.* 2001;97:2923-2931.
22. Verneris M, Ito M, Baker J, Arshi A, Negrin RS, Shizuru JA. Engineering hematopoietic grafts: purified allogeneic hematopoietic stem cells plus expanded CD8+ NK-T cells in the treatment of lymphoma. *Biol Blood Marrow Transplant.* 2001;7: 532-542.
23. Schmidt-Wolf IG, Finke S, Trojaneck B, et al. Phase I clinical study applying autologous immunological effector cells transfected with the interleukin-2 gene in patients with metastatic renal cancer, colorectal cancer and lymphoma. *Br J Cancer.* 1999;81:1009-1016.
24. Takayama T, Sekine T, Makuuchi M, et al. Adoptive immunotherapy to lower postsurgical recurrence rates of hepatocellular carcinoma: a randomised trial. *Lancet.* 2000;356:802-807.